Apoptotic cell death is involved in normal and pathologic cardiac function. Apoptosis is pivotal in normal developmental cardiogenesis and yet unregulated cardiomyocyte loss via apoptosis contributes to cardiac degeneration in heart failure. There is emerging evidence that distinct populations of cardiomyocytes may exhibit differential susceptibility to apoptosis. We showed that alternative splicing of the cardiac ryanodine receptor (RyR2) modulated the susceptibility of cardiomyocytes to Ca^{2+}-linked apoptosis (George et al., (2007)). Specifically, a 24bp splice variant (encoding VTGSQRSK inserted distal to residue 3715 in human RyR2) suppressed homeostatic and agonist-evoked Ca^{2+} fluxes in cardiomyocytes. We proposed that alternative splicing of RyR2 may represent a mechanism for 'tuning' pro- and anti-apoptotic Ca^{2+} fluxes. Our data supports the concept that the 24bp splice insertion stabilises RyR2-mediated Ca^{2+} release, and we tested this hypothesis using single channel and cellular approaches. In single channel experiments using CHAPS-solubilised, sucrose gradient-enriched preparations incorporated into PE bilayers, we investigated the effects of synthetic peptides containing VTGSQRSK on the Ca^{2+} sensitivity of recombinant human RyR2 channels devoid of alternatively spliced insertions ($RyR2^{-/-}$). In a complementary approach we used confocal imaging to determine the effects of these peptides on Ca^{2+} handling in living cardiomyocytes. Peptides were injected into the nucleus and cytoplasm of synchronously-coupled HL-1 cells (that exclusively express $RyR2^{-/-}$) and cellular Ca^{2+} fluxes were analysed using the multi-parametric **S**ynchronicity-**A**mplitude-**L**ength and **V**ariability of **O**scillation (SALVO) program that describes the 'contractile' and 'non-contractile' aspects of cellular Ca^{2+} handling. In both approaches, peptides encoding a splice insertion that did not protect cells from Ca^{2+}-linked apoptosis (FAIDSLCGFG), or scrambled sequences were used as controls. Our data shows the utility of these complementary approaches in determining the mechanistic basis of altered cellular Ca^{2+} handling mediated by alternative splicing of RyR2.

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Use Of *Shaker* B K^+ Channel NH_2-inactivation Peptides To Probe The Ryanodine Receptor Ca^{2+} Release Channel Pore

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Inactivation of K^+ channels occurs by the interaction of the pore with NH_2-terminal sequences. Synthetic peptides corresponding to these sequences were applied to the purified mouse cardiac ryanodine receptor type 2 (RyR2) and single channel activity was recorded in planar lipid bilayers. The wild type (WT) *Shaker* B NH_2-peptide MAAVAGLYGLGEDRQHRKKQ induced a block of the open RyR2 channel in a concentration and voltage-dependent manner, but also when the channel displayed a ryanodine-modified state [Mead *et al.*, J. Membrane Biol., 1998]. In the latter condition, in a 200 mM KCl buffer when the holding potential was at +50 mV, the open probability decreased by a factor of 2 ($p=0.001$, $n=10$ channels for control and $n=5$ for WT peptide) mainly due to a 4-fold reduction ($p<0.01$, $n=10$ channels for control and $n=5$ for WT peptide) of the mean open time in the presence of 20 μM WT peptide. It is noteworthy that the peptide at 20 μM had no effect in a 600 mM KCl buffer, suggesting the importance of charged residues in the blocking effect on RyR2. Furthermore we designed a mutant peptide where the amino acid Alanine in positions 3 and 5 was replaced by Glutamine residues, giving a less hydrophobic feature to the peptide. The mutant peptide at 20 μM in a 200 mM KCl buffer was less effective than the WT peptide, while showing a different mechanism of action. These findings are consistent with the idea that RyR2 contains well organised charged and hydrophobic residues in its conduction pathway and that they may play a general role in the ion translocation mechanisms of the sarcoplasmic reticulum Ca^{2+} release channel.

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Imperatoxin A, A Calcin Toxin From *Pandinus Imperator* Scorpions, Ablates Calcium Sparks In Permeabilized Cells

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Imperatoxin A (IpTxa) is a 33 amino acid peptide toxin from the African scorpion *Pandinus imperator*. Its definitive structural and functional characteristics designate it as a member of the calcin family, a unique group of peptide toxins, which to date includes Hemicalcin (HCa), Hadrucalcin (HdCa), IpTxa, Maurocalcin (MCa), and Opicalcins 1 & 2 (Opi 1 & 2). Calcins are unified by 1) a compact, globular structure containing an inhibitor cystine knot, 2) positive net charge, 3) the ability to activate ryanodine receptors (RyR) with high affinity and selectivity, and 4) presumed or confirmed cell-penetrating capabilities. Previous studies have demonstrated the ability of MCa to carry a fluorescent cargo to the interior of intact cells, and we have used a similar method to confirm that IpTxa is also a cell-penetrator. In addition, we have demonstrated IpTxa's ability to transiently enhance calcium release from the sarcoplasmic reticulum (SR) of intact cells. In the current study, we used confocal microscopy to explore the effects of IpTxa on saponin-permeabilized cells. Perfusion of 10nM IpTxa on cells resulted in a dramatic reduction of sparks to as low as 1% of control within two minutes. This effect was even more pronounced and rapid in the presence of 50nM toxin, with complete ablation of sparks in less than 1 minute. At the same time, mean fluorescence is often increased in our scans relative to control, although rapid application of caffeine reveals that SR calcium content is retained. Our results suggest that IpTxa depletes calcium from the SR, a hypothesis that would explain not only the ablation of sparks, but also the transient nature of enhanced calcium release in intact cells observed upon perfusion with IpTxa.

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Block Of Mouse Cardiac Ryanodine Receptor (mRyR2) By hERG Blocking Agents

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This study investigates the novel block from the cytosolic face of mRyR2 by three hERG blocking agents. For this purpose, astemizole (A), terfenadine (T) and chloroquine (C) were investigated based on their different affinities of hERG block (IC_{50} = 0.9 nM, 56–204 nM and 2500 nM) and differences in their proposed mechanisms of action at the cytosolic face of ion channels. As well as exhibiting high affinity block of hERG, these blockers exhibit half maximal block of multiple ion channel types at concentrations exceeding 1×10^{-6} M. Whilst mRyR2 lacks the key residues (Y652 and F656) involved in high affinity block of hERG it does possess a wide pore containing a high density of hydrophobic residues. Therefore the potential exists for mRyR2, like hERG, to be blocked by a diverse range of compounds with high affinity. The aim of this study is to examine the properties/mechanisms of mRyR2 block to identify areas within mRyR2 that may be involved in drug binding. Similar to their action in non-hERG ion channels, these compounds block mRyR2 within the micromolar range (IC_{50}; (A) - 16.12 ± 2.12 μM; (T) - 30.04 ± 4.81 μM and (C) - 17.73 ± 5.14 μM; n = 5) at + 50 mV. Whilst all blockers reduce the single channel current amplitude to ~ 95 % of control levels, astemizole and terfenadine induce additional blocking states, whilst also allowing channel closure during periods of block. Chloroquine exhibits briefer blocking events, indicating a faster off rate and no additional reduced conductance states observed. This work was funded by the British Heart Foundation.

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Cardiac Ca2+ Release Channel/RyR2 -Molecular Mechanism Of Green Tea Extract epigallocatechin-3-gallate

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Consumption of green tea has been found to closely correlate with a lower risk of heart disease and reduced cardiovascular mortality. Extracted from green leaves, a group of catechin polyphenols appears to be responsible for these beneficial cardiovascular effects. Studies have shown that epigallocatechin-3-gallate (EGCG), one of the most abundant and potent catechins, can modulate myocardial contractility through some unknown mechanisms. Recent evidence suggests a role for Na^+/H^+ and Na^+/Ca^{2+} exchangers in the ionotropic effects of EGCG in rat heart. Here we report that cardiac Ca^{2+} release channel - RyR2 serves as a possible molecular target of EGCG that may be responsible for amplified Ca^{2+} transients, enhanced ventricular contractility and increased cell shortening in cardiac myocytes. In single isolated mouse ventricular myocytes EGCG (5μM) increased peak systolic Ca^{2+} transient amplitude, increased decay of the Ca^{2+} transient, increased fractional shortening and increased contraction and relaxation velocities. To assess the direct interaction of EGCG with RyR2, both purified and membrane-bound RyR2 single channels were reconstituted in planar lipid bilayers. In the presence of 0.5μM EGCG, the open probability (Po) increased from Po=0.012 in the control state to Po=0.387. Using [^{3}H]ryanodine as a probe to assess channel conformational states, our data further indicate that (1) EGCG dose-dependently enhanced junctional SR membrane-bound RyR2 channel activity and (2) EGCG increased Ca^{2+}-induced Ca^{2+} activation of RyR2 (EC_{50} = 7.0 and 1.7 μM for control and EGCG, respectively). In summary, our data are consistent with previous reports for positive inotropic and lusitropic effects by EGCG on heart cells. In addition, our data clearly supports a role of EGCG in modulating cardiac E-C coupling through interacting with RyR2 channel complex. Supported by K01 AR51519 to GR and P01 AR52354.